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# Transport of species in a bio-reactor for bone tissue engineering

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One of the most important issue in tissue engineering is the development of bio-reactors in which the best conditions for cells growth can be created and maintained for the time necessary to produce the tissue. These conditions include homogeneous flow distribution to obtain sufficient transport of nutrients (mainly glucose and oxygen) and efficient removal of catabolites together with low levels of shear stress at the surface of internal scaffolds where the cells are supposed to grow.
In this work we simulate numerically the flow and transport of nutrients in a bio-reactor used to produce bone tissues which may then be implanted into patients to repair jaw defects. We characterize the flow distribution and the shear stress distribution at the surface of the scaffold. We solve for the transport of nutrients and we simulate the effect of local nutrients concentration at the surface of the scaffold on the growth rate of cellular tissues. Results of the numerical simulation can be used to improve the design of the bio-reactor and to identify the flow conditions which maximize cell growth.

## 1. Introduction

Bio-reactors used for tissue engineering should be properly designed to promote the in vitro growth of cells on scaffolds. In the optimal design, the gas transfer and the nutrient delivery to cells attached to the scaffold should be promoted yet avoiding the detachment of cells due to large shear stress at the wall (Hutmacher, 2000, Hutmacher et al., 2004). According to some experiments, the shear stress may also contribute to cell metabolism (Singh et al., 2005) thus enhancing/reducing the growth rate of cellular matter. It is rather difficult and costly to identify the optimal design of a bio-reactor/scaffold system performing experiments on alternative configurations. Numerical analysis offers as a valuable alternative for the ``virtual" testing of scaffold/bioreactor systems, which can give useful indication on bio-reactor design before the experimental testing phase. This approach has been used by Singh et al. (2005) to characterize the flow and cellular growth rate in a bi-axial bio-reactor system working under different operating conditions. Boschetti et al. (2006) and Porter et al. (2005) performed numerical analyses to quantify the variation in the shear stress acting

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on 3-D scaffolds as a function of flow rate and scaffold characteristics (pore size and porosity). Ma et al. (2007) investigated the fluid flow, shear stress and nutrient distribution within a scaffold to verify if the bio-reactor design and working conditions were suitable for the development of haematopoietic cells. In this work we use a numerical approach to characterize the flow, shear stress distribution and nutrient transport in a bio-reactor used for bone tissue engineering with the final aim of developing guidelines for the optimal design of bioreactors.

## 2. Configuration under study

Figure 1 shows a sketch of the bio-reactor investigated in this work. The bio-reactor is made of a 12x12 cm rectangular box containing a 3-D scaffold. The scaffold is made of cylindrical fibers (d=0.5 mm) arranged in a 3-D matrix built around an hollow cylinder which holds the scaffold in place inside the bio-reactor and gives the mechanical resistance to the grown bone tissue for tissue re-modelling before implantation. The bio-reactor is fed from one side by a pipe (4 mm diameter) delivering a water solution containing glucose and oxygen. The solution (Q=1 $cm^3$/min) flows through the feeding pipe to four smaller pipes (distributing pipes, 1 mm diameters) and then into a conical volume which distributes the fluid around the scaffold. The configuration is symmetrical at the other end of the box.

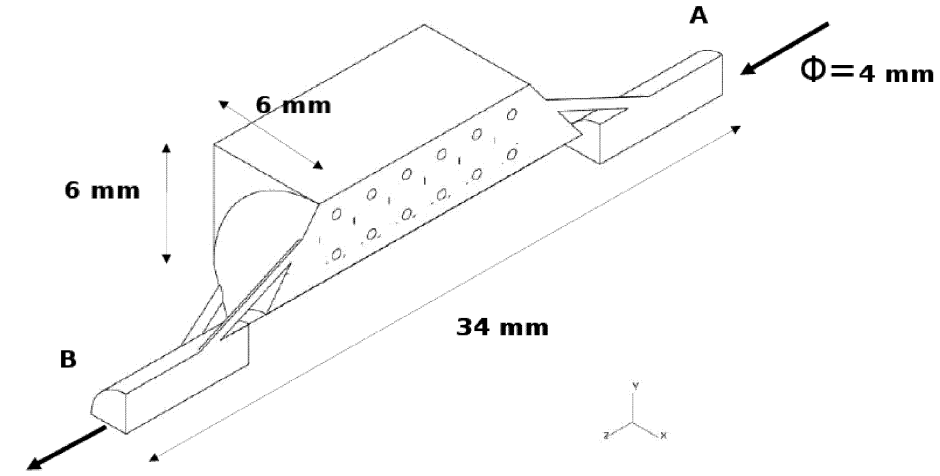


*Figure 1: sketch of the bioreactor .*

The solution of water, oxygen and glucose fed to the bio-reactor is supposed to deliver the nutrient necessary to promote the cellular growth and to efficiently remove catabolites which may form from the cellular growth. In this bio-reactor configuration, oxygen may become a limiting factor for cellular growth: while the amount of glucose fed to the cells can be increased with no limitations, the amount of oxygen available in the flow is controlled by the saturation concentration and by the flow rate. Yet, by increasing the flow rate, a larger shear stress can be produced at the scaffold walls which may cause the detachment of cellular matter from the scaffold.

## 3. Methodology

In this work, we use numerical simulations to characterize the flow, the shear stress distribution and the transport of nutrient inside the bio-reactor.
In the conditions investigated, the flow is laminar: the Reynolds number in the inlet pipe is Re~5, the average velocity in the bio-reactor is 1.15 $10^{-4}$ m/s and the fiber Reynolds number is $Re_{cyl}$ ~5 $10^{-2}$. Navier Stokes equation can be solved for the flow domain to calculate the velocity field inside the bio-reactor, the pressure drop across the bio-reactor and the shear stress distribution at the walls of the scaffold. The transport equation can be solved to calculate the local concentration of nutrients and the cellular growth. Given the axial symmetry of the domain, in this work only 1/8 of the scaffold has been simulated. We used a commercial finite volume solver of N-S equations (Star-CD) to solve for the flow. Physical boundary conditions are: (I) fixed flow rate at the inlet section A, (ii) no slip at walls of scaffold and bioreactor, (iii) outlet conditions at section B (see Figure 1), (iv) symmetry conditions at the other surfaces.
For the transport/reaction of nutrients/catabolites, a bio-reaction kinetic model has been implemented to simulate the cellular growth at the fiber surface. Figure 2 shows a sketch of the bio-reactions implemented in our simulation.

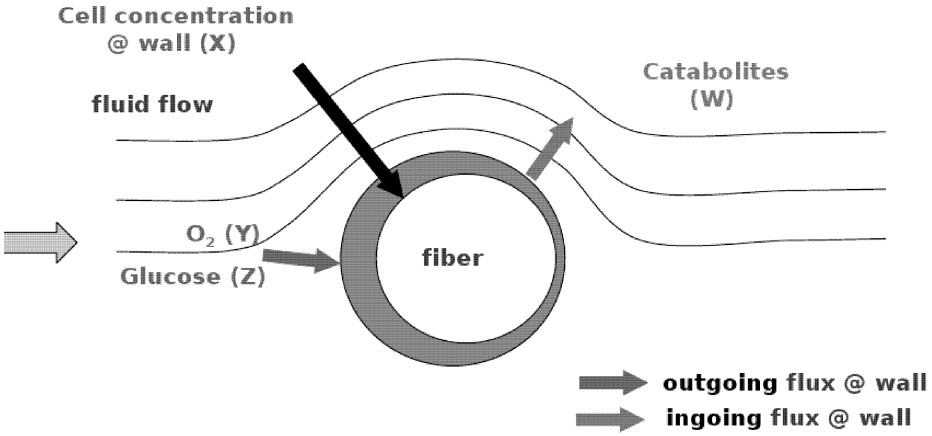


*Figure 2: sketch of the bio-reactions used to simulate cellular growth.*

Bio-reactions can be summarized by the following 'symbolic' equation:
Glucose + Oxygen → Cellular growth + Catabolites
which can be used to calculate ingoing/outgoing fluxes of transported species at the fiber surface. Assuming that the initial concentration of cells at the surface of the scaffold is known at the starting time (x=x(θ,t=0)), the variation over time of the concentration of cells at the surface of each fiber of the scaffold will be a function of the local growth rate, which in turn depends on the local concentration of oxygen (y) and glucose (z) transported at the fiber surface. This equation can be written as:

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \mu \frac{y}{y+k} x$$

where μ and k are the parameters of the Monod type kinetics used to simulate cellular growth, y is the oxygen concentration (considered as a grow limiting factor). This equation is to be solved with:

$$\frac{\mathrm{d}y}{\mathrm{d}t} = -\frac{\mathrm{d}x}{\mathrm{d}t}\frac{1}{\eta_{x/y}} = -\mu\frac{y}{y+k}\frac{x}{\eta_{x/y}}$$

which describes the consumption of y per unit of mass of cells grown, $\eta_{x/y}$ being the reaction yield (grams of y consumed per 1 gram of cells grown). Similar equations representing the local consumption of glucose (z) and the local formation of catabolites (w) should be solved for glucose and catabolites. Cellular kinetics allows to calculate the fluxes across the fiber surface which are then used as boundary condition for the transport of species (oxygen, glucose and catabolites) in the bio-reactor domain.

## 4. Results

### 4.1 Flow field and shear stress distribution

Figure 3 shows the velocity field calculated in the bio-reactor.

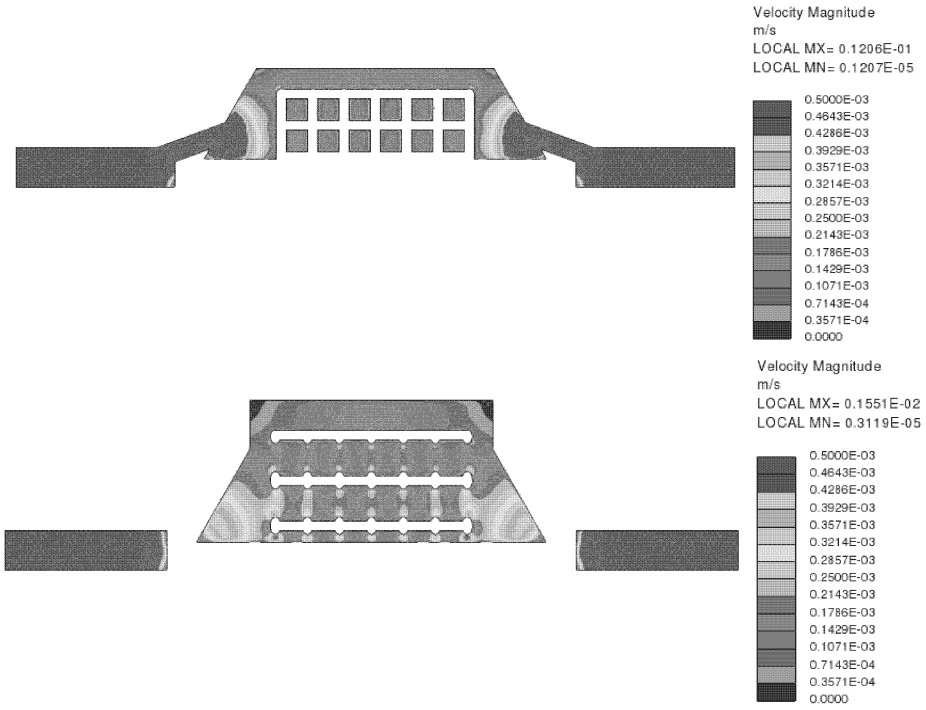


*Figure 3: velocity field inside the bio-reactor. The color scale shows velocity values in the range [0:0.5] mm/s.*

The upper section is taken across one distributing pipe; the lower section is taken in between two distributing pipes. The color scale is reduced to show velocity values in the lower range (flow inside the bio-reactor). As expected for a laminar flow at very low Reynolds number, the flow is symmetrical in the inlet and outlet side.
The flow velocity in the box is less than 0.1 mm/s inside the bio-reactor. Some dis-homogeneities exist in the flow distribution right at the exit of the four distributor pipes.
Figure 4 shows the shear stress distribution at the surface of the scaffold. Values are given in N/mm$^2$. The shear stress on the scaffold is sufficiently small to guarantee cellular adhesion to the wall. Yet, values of the shear stress change over three orders of magnitude, with the largest values for the inner row of fibres, near the inlet.

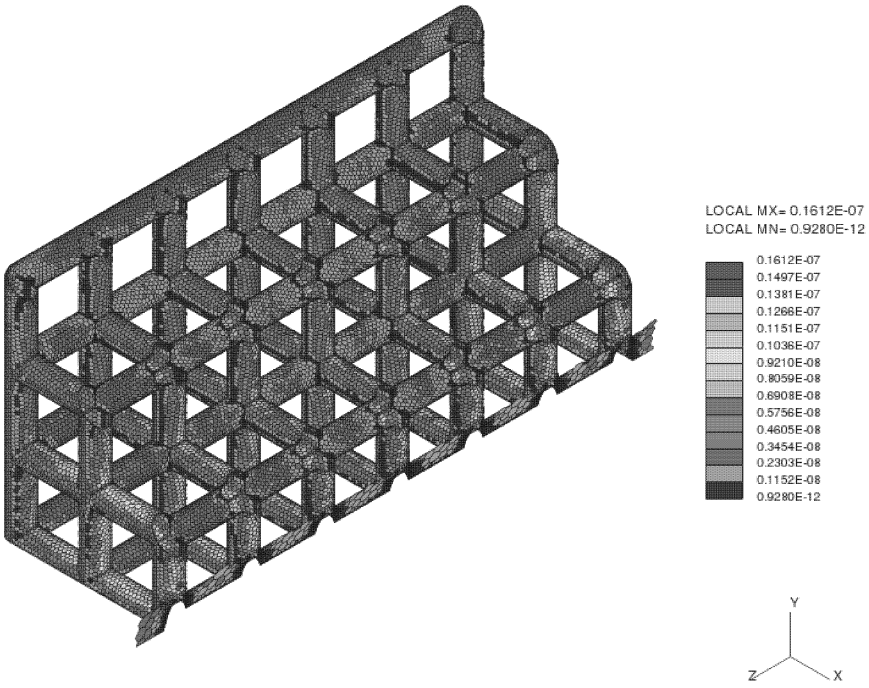


*Figure 4: shear stress at the scaffold wall. Values are given in N/mm$^2$.*

**4.2 Transport of nutrients and cellular growth**

Figure 5 shows the local concentration of catabolites calculated at the surface of different fibers of the scaffold. At this stage of the work, the results we show should be considered qualitatively. Some kinetic experiments are under way to evaluate the value of the constants of the kinetic model. The relevant results is that, even when the flow in the bio-reactor is fully developed, the local cellular growth rate (and catabolites production) may be significantly different from fiber to fiber. This may lead to the non homogeneous growth of cellular matter. A great attention should be paid to the gradual reduction of the oxygen concentration in the flow, which is the main responsible for this effect, when designing optimized scaffold/bio-reactors systems.

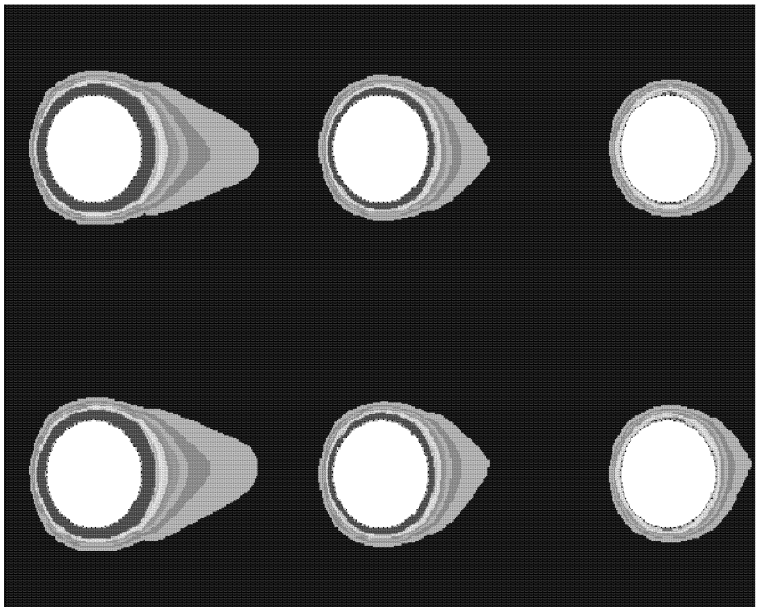

*Figure 5: concentration of catabolites around three fibres aligned along the flow. Cellular growth and catabolites may change significantly inside the scaffold.*

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